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Measurement of neural respiratory drive via parasternal intercostal electromyography in healthy adult subjects

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Abstract

Introduction: Neural respiratory drive, quantified by the parasternal intercostal muscle electromyogram (EMGpara), provides a sensitive measure of respiratory system load-capacity balance. Reference values for EMGpara-based measures are lacking and the influence of individual anthropometric characteristics is not known. EMGpara is conventionally expressed as a percentage of that obtained during a maximal inspiratory effort (EMGpara%max), leading to difficulty in applying the technique in subjects unable to reliably perform such manoeuvres.

Aims: To measure EMGpara in a large, unselected cohort of healthy adult subjects in order to evaluate relevant technical and anthropometric factors.

Methods: Surface second intercostal space EMGpara was measured during resting breathing and maximal inspiratory efforts in 63 healthy adult subjects, median (IQR) age 31.0 (25.0 – 47.0) years, 28 males. Detailed anthropometry, spirometry and respiratory muscle strength were also recorded.

Results: Median (IQR) EMGpara was 4.95 (3.35 – 6.93) μ V, EMGpara%max 4.95 (3.39 – 8.65)% and neural respiratory drive index (NRDI, the product of EMGpara%max and respiratory rate) was 73.62 (46.41 – 143.92) arbitrary units. EMGpara increased significantly to 6.28 (4.26 – 9.93) μ V ($p < 0.001$) with a mouthpiece, noseclip and pneumotachograph *in situ*. Median (IQR) EMGpara was higher in female subjects (5.79 (4.42 – 7.98) μ V *versus* 3.56 (2.81 – 5.35) μ V, $p = 0.003$); after controlling for sex neither EMGpara, EMGpara%max or NRDI were significantly related to

anthropometrics, age or respiratory muscle strength. In subjects undergoing repeat measurements within the same testing session (n=48) or on a separate occasion (n=19) similar repeatability was observed for both EMGpara and EMGpara%max. Conclusions: EMGpara is higher in female subjects than males, without influence of other anthropometric characteristics. Reference values are provided for EMGpara-derived measures. Expressing EMGpara as a percentage of maximum confers no advantage with respect to measurement repeatability, expanding the potential application of the technique. Raw EMGpara is a useful marker of respiratory system load-capacity balance.

Introduction

Measurement of neural respiratory drive (NRD), the output of brainstem respiratory centres, provides a marker that reflects the balance between the physiological load on the respiratory system and the capacity of the respiratory muscles. Such a measure may provide a useful composite index of overall respiratory system derangement in the presence of disease, or during physiological studies. NRD cannot be quantified at source in human subjects; instead, the neural input to selected important respiratory muscles in the form of the electromyogram (EMG) can be used as an index of NRD. As the primary muscle of inspiration, the diaphragm remains the best option for measurement of NRD and such measures reflect lung disease severity in chronic obstructive pulmonary disease and cystic fibrosis (Jolley *et al.*, 2009; Reilly *et al.*, 2011). Obtaining accurate, uncontaminated EMG signals from the diaphragm however requires the passage of an oesophageal catheter, rendering the technique unsuitable for widespread use.

The parasternal intercostal muscles are obligate muscles of inspiration, recruited in tandem with the diaphragm and displaying similar patterns of activity. Although early research in both animal and human subjects used needle EMG techniques (De Troyer & Sampson M.G., 1982; De Troyer, 1984; Decramer & De Troyer, 1984), the anatomical location of the parasternal intercostal muscles and the lack of active overlying musculature allows their electrical activity to be assessed using surface electrodes. The potential utility of parasternal intercostal muscle EMG (EMGpara) measurements as a marker of NRD has been established in both clinical and laboratory settings, in health and disease. Relationships between EMGpara and disease severity in obstructive lung diseases have been demonstrated (Maarsingh *et al.*, 2002; Reilly *et al.*, 2011; Steier *et al.*, 2011; Reilly *et al.*, 2012), along with increases in EMGpara in response to externally-imposed respiratory load in healthy subjects (Reilly *et al.*, 2013). Differences between healthy individuals and those with lung disease have also been demonstrated (Steier *et al.*, 2009; Murphy *et al.*, 2011; Reilly *et al.*, 2011; Steier *et al.*, 2011), *via* comparison to small groups of matched individuals.

As with other EMG measurements, EMGpara has been expressed as a percentage of the signal obtained during a maximal inspiratory effort (EMGpara%max) in order to minimise inter-individual and inter-occasion variability arising from differences in muscle-electrode distance and electrode placement. Obtaining reliable and reproducible maximal inspiratory efforts may not however be possible in all subject groups, such as young children or individuals with reduced levels of consciousness. Reproducibility of EMGpara%max measurements has been demonstrated (Murphy *et al.*, 2011; Reilly *et al.*, 2011), but these studies have mainly been undertaken in small groups of healthy individuals experienced in respiratory manoeuvres. It is not known whether naïve subjects are as able to reliably perform such maximal respiratory efforts reproducibly.

Robust reference data for EMGpara in large groups of healthy adult subjects, such as those available for EMGdi (Jolley *et al.*, 2009), are not available and no previous studies have, to our knowledge, investigated the influence of anthropometric characteristics on the EMGpara signal. Such data would facilitate standardised interpretation of EMGpara measurements made in both research and clinical populations.

The aims of the current study were therefore:

- To measure EMGpara in a large, unselected cohort of healthy adults
- To determine anthropometric factors influencing EMGpara, thereby deriving reference data for EMGpara
- To evaluate reproducibility of the raw (EMGpara) and normalised (EMGpara%max) signals.

Methods

Subjects

The study conformed to the requirements of the Declaration of Helsinki. Ethical approval was obtained from the Biomedical Sciences, Dentistry, Medicine and Natural Mathematical Sciences Research Ethics Subcommittee of King's College London (reference number BDM/13/14-87). All participants provided informed written consent prior to commencing the study.

Participants were recruited via local intranet advertisements, posters within the hospital, and via word of mouth. Subjects were eligible for inclusion if they were aged over 18 years of age, a non-smoker and had no history of respiratory, cardiac or neurological disease. Any subjects demonstrating cough or coryzal symptoms at the time of testing were excluded. Subjects with abnormal spirometry were excluded (further details below). Testing was conducted in a climate-controlled room maintained at 23 degrees centigrade. Measurements were performed at least two hours following food or drink consumption.

Spirometry

All participants performed spirometry with a hand-held electronic spirometer incorporating a Fleisch-type pneumotachograph meeting international guidelines (In2ative, Vitalograph Ltd., Buckingham, England). Spirometry was performed in accordance with ATS/ERS criteria (Miller *et al.*, 2005); efforts were continued until the subject had produced three technically-acceptable forced vital capacity manoeuvres from which the highest two values for both FEV₁ and FVC were within 0.15l. The highest values for both FEV₁ and FVC were reported. Prior to each testing session, the accuracy of the spirometer was verified using a 3 litre gas syringe. All values were corrected to BTPS conditions and expressed as standardised residuals ("z scores") relative to the predicted values for age, height, sex and ethnicity described by the Global Lung Initiative (Quanjer *et al.*, 2012). Any subjects demonstrating an abnormal FVC or FEV₁ (greater than 1.96 z-scores below the predicted value) were excluded from further participation.

Anthropometrics and body composition analysis

Subjects were asked to remove footwear and any heavy clothing. Height was measured using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crymych, UK) with a resolution of 1mm and a range of 600-2100mm, validated daily with a certified one-metre bar. Weight was measured using an electronic scale (HR Person Scale, Marsden Ltd, Henley-on-Thames, UK) with a resolution of 50g and maximum capacity of 300kg, validated daily with calibrated weights. Body mass index was calculated by dividing the subject's weight by the square of their height in metres.

Waist and hip circumference were measured in centimetres using a non-extensible measuring tape placed around the narrowest part of the abdomen (or, if unclear, the midpoint between the inferior border of the tenth rib and the iliac crest) and around the level of the greater trochanter respectively. Waist-hip ratio was calculated as a measure of central obesity. Neck circumference was measured in centimetres at the level of the laryngeal prominence with the tape orientated perpendicular to the long axis of the neck, and was used as a measure of upper body adiposity.

Bioelectrical impedance was used to calculate body fat percentage using a Quadscan 4000 (Bodystat Ltd, Douglas, Isle of Man) in accordance with the manufacturer's guidelines. The subject lay supine for five minutes prior to measurement to allow body water redistribution to occur. After skin preparation using an alcohol swab, two electrodes were placed on the dorsum of the right foot over the 2nd metatarsophalangeal joint and between the lateral and medial malleoli of the ankle, and two over the dorsum of the right hand at the level of the 2nd metacarpophalangeal joint and the midpoint of the wrist orientated transversely across the joints. Fat and lean mass and fat percentage were recorded.

Electromyography

Skin was prepared in accordance with international guidelines for EMG recording (Hermans *et al.*, 2000). Initially the skin was scrubbed with an abrasive gel (Nuprep, Weaver and Company, Aurora, USA) to remove dead skin cells and excessive sebum in order to minimise electrode-skin impedance. Any remaining gel and sebum was

then removed with an alcohol-impregnated wipe before self-adhesive silver-silver-chloride electrodes were applied (Kendall Arbo, Tyco Healthcare, Neustadt/Donau, Germany). Electrodes were positioned in the second intercostal space bilaterally, 3cm lateral to the sternum, with a reference electrode placed on the clavicle or the acromion process of the scapula.

Signals were amplified (gain 1,000) and band-pass filtered between 10 and 2,000Hz, with an additional adaptive mains filter to minimise mains frequency interference, using a CED 1902 biomedical amplifier (Cambridge Electronic Design, Cambridge, UK). EMG signals were acquired (PowerLab 8/35, ADInstruments, Sydney, Australia) and displayed on a laptop computer running LabChart software (Version 7.2, ADInstruments Pty, Colorado Springs, USA) with analogue to digital sampling at 10kHz. Both amplifier and analogue-to-digital convertor were earthed to suitable points in the laboratory. Additional digital post-acquisition band-pass filtering between 20-1,000Hz was applied via the LabChart software to isolate the frequencies of interest. Data were displayed as both raw EMG and as a root mean square (RMS) trace, using a moving average window of 50ms (Ives & Wigglesworth, 2003).

EMGpara was recorded during tidal breathing. Subjects were seated upright in a chair with back supported, arms placed on armrests and feet flat on the floor to minimise trunk postural activity. Subjects were instructed to remain still and not to speak throughout the recording period. EMGpara was also recorded during maximal inspiratory efforts, as described by other authors (Jolley *et al.*, 2009; Reilly *et al.*, 2011; Steier *et al.*, 2011), in order to allow normalisation of the resting signal to maximal EMGpara activity. EMGpara was recorded during sniff nasal inspiratory pressure (SNIP) and maximal inspiratory pressure (PImax) manoeuvres, during inspiration to total lung capacity (TLC) and during a single fifteen-second maximum voluntary ventilation (MVV). The highest EMGpara recorded during each maximal manoeuvre was identified and reported. An example of recorded EMGpara data is shown in Figure 1.

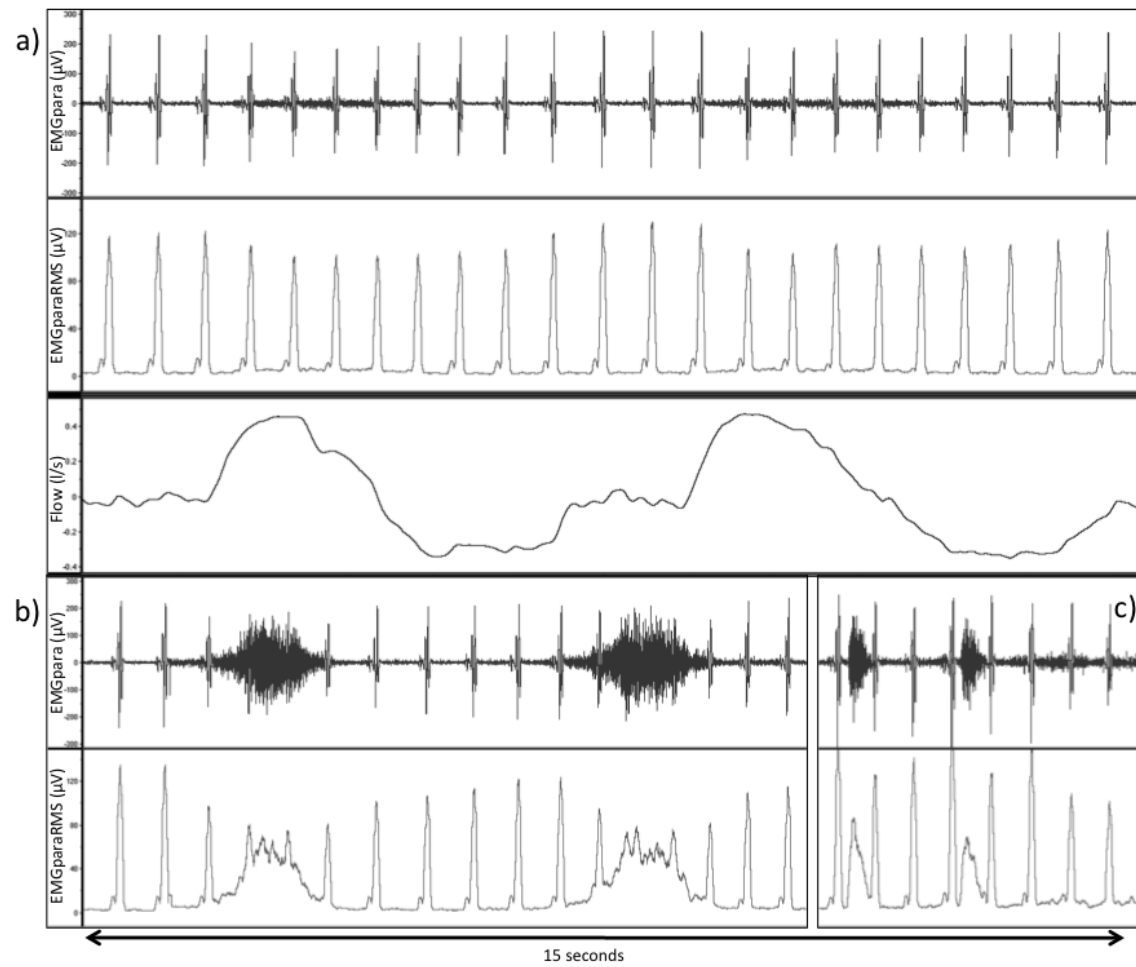


Figure 1: Example EMGpara recordings during tidal breathing (1a), inspiration to total lung capacity (1b) and maximal sniff manoeuvre (1c) from a 32 year-old female subject, showing raw EMGpara trace (“EMGpara”), root mean square (“EMGparaRMS”) and respiratory flow (1a only, inspiration positive).

Following visual inspection of the traces to ensure absence of artifact from external electromagnetic interference or cross-talk from other musculature, inspiratory EMGpara activity, occurring between QRS complexes, was highlighted and the peak RMS EMGpara calculated. The mean peak RMS EMGpara per breath was calculated over the final minute of each recording period. EMGpara was expressed both as a raw RMS value in microvolts (μV) termed “EMGpara” and as a percentage of the highest RMS EMGpara value recorded during the maximal inspiratory efforts (“EMGpara%max”). The neural respiratory drive index (NRDI, expressed in %breaths/min (%BPM)) was also calculated as the product of EMGpara%max and respiratory rate (Murphy *et al.*, 2011).

Measurement of respiratory flow, volume and airway pressure

In order to facilitate identification of respiratory phase, respiratory flow was measured using a pneumotachograph (4500 series, range 0-160l/min, Hans Rudolph Inc, Kansas City, USA) attached to a flanged mouthpiece. Subjects wore a noseclip. The pressure drop across the pneumotachograph was measured using a differential pressure transducer (Spirometer, AD Instruments, Castle Hill, Australia). Airway pressure was measured during manoeuvres for respiratory muscle strength assessment using a second differential pressure transducer (MP45, Validyne, Northridge, USA) and associated carrier amplifier (CD280, Validyne, Northridge, USA). The system had a combined frequency response of 42Hz, in line with international recommendations (American Thoracic Society & European Respiratory Society, 2002). The flow and airway pressure signals were acquired by the data acquisition system with 100Hz analogue to digital sampling and displayed alongside the EMGpara on the LabChart software. Volume was derived *via* digital integration of the flow signal.

Measurement of respiratory muscle strength

Inspiratory muscle strength was measured in accordance with ATS/ERS criteria (American Thoracic Society & European Respiratory Society, 2002). Sniff nasal inspiratory pressure (SNIP) was measured using a bung placed in the most patent nostril and attached to the differential pressure transducer *via* a non-compressible polyethylene catheter. Subjects were asked to perform a series of short, sharp maximal sniffs, repeated until three sniffs with peak pressures of within 5% were achieved with the highest pressure reported. Quality assurance criteria as per Uldry and Fitting were applied (Uldry & Fitting, 1995).

Maximal inspiratory pressure (P_Imax) was assessed by asking the subject to perform a maximal inspiratory effort from functional residual capacity against a closed valve. A small leak (2mm internal diameter) was incorporated into the apparatus to prevent recruitment of orofacial musculature and ensure an open glottis. P_Imax was calculated as the highest mean pressure over one second (American Thoracic Society & European Respiratory Society, 2002). The highest value of three reproducible efforts was reported.

Protocol

Subjects attended the laboratory on a single occasion, with a subset returning for repeat measurements to investigate inter-occasion reproducibility of the technique. On initial attendance, subjects undertook anthropometric and spirometric measurements, followed by assessment of bioelectrical impedance. Electrodes were then applied and EMGpara recorded. Adopting this order of testing ensured the subjects had sufficient rest and there was no influence of the maximum efforts required for spirometry on the EMGpara data.

EMGpara was recorded during five minutes of resting breathing without a mouthpiece, followed by a further five minutes with the mouthpiece, pneumotachograph and noseclip *in situ*. Maximal respiratory manoeuvres were

then performed in the following order: inspiration to TLC, SNIP, PImax and MVV. If subjects consented, electrodes were removed, skin re-prepared and all EMGpara measurements repeated. In the subjects able to return, testing was repeated at least seven days after the initial testing occasion at which point all EMGpara measurements were performed.

Statistical analysis

Statistical analysis was undertaken using SPSS Version 22 (IBM, USA). Normality of data distribution was assessed using D'Agostino Pearson testing. Inspection of the data demonstrated multiple variables to have a non-normal distribution and non-parametric statistical testing was therefore used throughout, with data expressed as median (IQR). Inter-occasion agreement was assessed using Wilcoxon's matched pairs tests and Bland-Altman plots (Bland & Altman, 1986). Relationships between variables were assessed using Spearman's correlation coefficients. Differences between groups were assessed using Mann-Whitney U test and within-subject differences using the related samples Wilcoxon signed rank test. Significance was accepted at $p < 0.05$.

The study was conducted using a convenience sample and no a priori power calculation was undertaken. The acquired sample size was sufficient to accurately detect a correlation coefficient of 0.4 with 90% power at the 5% level.

Results

A total of 79 subjects were recruited, from whom 63 acceptable data sets were obtained. Of the 16 participants excluded, 14 were due to abnormal findings on spirometry and two due to excessive ambient electrical interference on the EMG recording. Data regarding intra-occasion reproducibility were available in 48 subjects, with inter-occasion reproducibility data obtained from 19 participants. Subject characteristics are shown in Table 1.

	All subjects (n=63)	Intra-occasion measurements available (n=48)	Inter-occasion measurements available (n=19)
Age (years)	31.0 (25.0 – 47.0)	31.0 (22.3 – 47.0)	27.0 (24.0 – 43.0)
Sex (M : F)	28 : 35	23 : 25	9 : 10
Height (cm)	168.0 (164.0 – 176.0)	168.8 (163.3 – 176.0)	170.0 (162.0 – 176.0)
Weight (kg)	66.5 (58.3 – 79.0)	69.9 (60.4 – 83.7)	69.5 (60.0 – 76.0)
Body mass index (kg.m ⁻²)	23.9 (20.9 – 25.7)	25.0 (21.3 – 26.1)	24.5 (20.8 – 25.5)
Waist-hip ratio	0.81 (0.76 – 0.87)	0.83 (0.77 – 0.88)	0.81 (0.74 – 0.88)
Neck circumference (cm)	34.5 (31.5 – 38.5)	36.0 (32.0 – 39.0)	34.0 (31.0 – 38.0)
Fat-free mass (%)	76.1 (70.7 – 80.0)	76.1 (69.0 – 79.8)	76.1 (67.7 – 82.2)
FEV ₁ (z score)	-0.3 (-0.7 – 0.1)	-0.27 (-0.84 – 0.08)	-0.30 (-0.65 – 0.14)
FVC (z score)	-0.1 (-0.6 – 0.5)	-0.14 (-0.53 – 0.57)	-0.04 (-0.34 – 0.65)
FEV ₁ /FVC ratio (z score)	-0.4 (-1.0 – 0.2)	-0.43 (-0.98 – 0.15)	-0.33 (-1.02 – 0.18)
Ethnicity (n (%))	White: 53 (84.1%) Black: 3 (4.7%) Asian: 7 (11.1%)	White: 39 (81.2%) Black: 3 (6.3%) Asian: 6 (12.5%)	White: 17 (89.4%) Asian: 2 (10.6%)

Table 1 Demographic and anthropometric characteristics of study participants. Data are expressed as median (IQR).

Relationships with anthropometrics, spirometry and respiratory muscle strength and influence of sex

For the cohort overall, median (IQR) EMGpara was 4.95 (3.35 – 6.93) μ V, EMGpara%max was 4.95 (3.39 – 8.65)% and NRDl was 73.62 (46.41 – 143.92)%·BPM. Significant inverse relationships were observed between both EMGpara and EMGpara%max and height, weight, BMI and respiratory muscle strength (Table 2), with the exception of EMGpara and SNIP. Inverse relationships were also seen between both EMGpara and EMGpara%max and FEV₁ and FVC when expressed in litres, although these relationships were not retained when spirometric values were expressed relative to predicted. Age was not related to either EMGpara ($r=0.099$, $p=0.44$) or EMGpara%max ($r=-0.119$, $p=0.354$).

	EMGpara		EMGpara%max	
	Spearman's rho	p value	Spearman's rho	p value
Height	-0.459	<0.001	-0.627	<0.001
Weight	-0.440	<0.001	-0.492	<0.001
BMI	-0.383	0.002	-0.285	0.24
Plmax	-0.345	0.006	-0.335	0.007
SNIP	-0.225	0.077	-0.332	0.008
FEV ₁ (actual)	-0.365	0.003	-0.449	<0.001
FEV ₁ (z score)	0.144	0.261	0.011	0.932
FVC (actual)	-0.424	0.001	-0.577	<0.001
FVC (z score)	0.101	0.430	-0.013	0.916
FEV ₁ /FVC ratio (actual)	0.035	0.786	0.198	0.119
FEV ₁ /FVC ratio (z score)	0.19	0.885	-0.046	0.718

Table 2 Relationships between EMGpara and EMGpara%max and anthropometry, respiratory muscle strength and spirometric parameters

All EMGpara-derived variables were significantly higher in female subjects than males (Table 3). Although the male and female subjects were well matched with respect to age ($p=0.14$) and spirometric parameters expressed relatively to predicted (FEV₁, FVC and FEV₁/FVC ratio z-scores, $p>0.2$ for all), differences were observed in height, weight, BMI, measures of adiposity and respiratory muscle strength between men and women (Table 3). Due to these differences the analyses of relationships between EMGpara and anthropometrics, spirometry and respiratory muscle strength were repeated within-sex. Importantly, the relationships observed previously in the

whole cohort were not retained, suggesting other factors specifically relating to sex were the major determinants of EMGpara.

	Male	Female	p value
Height (m)	177.0 (174.1 – 182.8)	164.0 (161.0 – 167.6)	<0.001
Weight (kg)	78.6 (71.1 – 90.2)	60.7 (52.8 – 65.5)	<0.001
BMI (kg.m ⁻²)	25.5 (23.0 – 27.2)	21.7 (20.1 – 24.7)	<0.001
Waist-hip ratio	0.88 (0.84 – 0.91)	0.77 (0.70 – 0.80)	<0.001
Neck circumference (cm)	38.8 (37.0 – 40.0)	32.0 (31.0 – 33.3)	<0.001
Fat-free mass (%)	78.1 (74.8 – 83.3)	72.3 (66.0 – 78.4)	0.001
Plmax (cmH ₂ O)	102.2 (71.0 – 129.3)	68.9 (56.9 – 86.8)	0.001
SNIP (cmH ₂ O)	84.3 (63.3 – 113.6)	70.8 (52.5 – 87.9)	0.39
EMGpara (μV)	3.56 (2.81 – 5.35)	5.79 (4.42 – 7.98)	0.003
EMGpara%max (%)	3.80 (2.56 – 4.69)	7.66 (5.24 – 9.92)	<0.001
NRDI (%.BPM)	58.26 (36.93 – 71.73)	118.44 (73.36 – 165.41)	<0.001

Table 3 Sex differences in anthropometric variables, respiratory muscle strength and measures of NRD

Influence of mouthpiece

EMGpara was significantly higher (4.95 (3.35 – 6.93)μV *versus* 6.28 (4.26 – 9.93)μV, p<0.001), and respiratory rate significantly lower (16 (13 – 18) breaths/minute *versus* 13 (11 – 16)breaths/minute, p<0.001) with the mouthpiece *in situ*. The change in EMGpara when measuring respiratory flow using the mouthpiece, pneumtoachograph and nose clip was not, however, significantly related to the change in respiratory rate (r=-0.18, p=0.17). Median (IQR) NRDI was also

significantly higher with a mouthpiece *in situ* (73.6 (46.4 – 143.9)%.BPM versus 92.9 (50.0 – 150.9)%.BPM, $p=0.006$), suggesting larger tidal volumes with the mouthpiece *in situ*.

Predicted values for NRD

As sex was the primary determinant of EMGpara, reference values for EMGpara parameters with and without a mouthpiece *in situ* for each sex are proposed (Table 4). As several data sets demonstrated positive skewness, values for EMGpara variables were also log-transformed.

		Males	Females
No mouthpiece	EMGpara (μ V)	4.36 (3.57 – 5.16)	6.11 (5.22 – 6.99)
	EMGpara%max (%)	3.93 (3.17 – 4.68)	7.93 (6.61 – 9.26)
	NRDI (%.BPM)	60.95 (48.60 – 73.30)	128.23 (104.29 – 152.17)
	<i>log</i> EMGpara	1.38 (1.22 – 1.55)	1.72 (1.56 – 1.87)
	<i>log</i> EMGpara%max	1.26 (1.07 – 1.44)	1.95 (1.76 – 2.13)
	<i>log</i> NRDI	3.99 (3.80 – 4.18)	4.70 (4.49 – 4.91)
With mouthpiece	EMGpara (μ V)	5.90 (4.68 – 7.12)	7.70 (6.66 – 8.73)
	EMGpara%max (%)	5.18 (4.17 – 6.20)	10.00 (8.49 – 11.51)
	NRDI (%.BPM)	64.33 (50.83 – 77.83)	146.82 (118.34 – 175.31)
	<i>log</i> EMGpara	1.64 (1.44 – 1.84)	1.96 (1.82 – 2.11)
	<i>log</i> EMGpara%max	1.52 (1.31 – 1.72)	2.19 (2.02 – 2.37)
	<i>log</i> NRDI	4.00 (3.76 – 4.24)	4.83 (4.62 – 5.04)

Table 4 Reference values for EMGpara data in male and female subjects. Data are shown as mean (95% CI).

Repeatability of EMGpara

Intra-occasion repeatability of EMGpara was assessed in 48 subjects (Table 5).

Although there were no differences in EMGpara%max and maxEMGpara between measurements made within the same testing session, EMGpara was significantly lower on second measurement ($p=0.015$). Bland-Altman analysis demonstrated a small bias towards a higher EMGpara ($0.63\mu\text{V}$), EMGpara%max (0.67%) and maxEMGpara ($3.05\mu\text{V}$) on the first occasion.

The inter-occasion repeatability was also assessed in 19 subjects with a median (IQR) of 9 (7 -13) days between occasions (Table 5). No significant differences were observed in any measure and Bland Altman analysis indicated negligible bias between occasions for EMGpara ($0.21\mu\text{V}$), EMGpara%max (0.24%) and maxEMGpara ($0.81\mu\text{V}$).

		First measurement	Second measurement	p value
Intra-occasion (n=48)	EMGpara (μV)	5.02 (3.38 – 6.92)	4.40 (3.05 – 6.04)	0.015
	EMGpara%max (%)	4.92 (3.67 – 8.12)	4.71 (3.41 – 7.15)	0.14
	maxEMGpara (μV)	95.36 (68.54 – 124.60)	97.24 (68.04 – 112.38)	0.712
Inter-occasion (n=19)	EMGpara (μV)	5.20 (3.45 – 6.91)	5.17 (3.48 – 7.28)	0.546
	EMGpara%max (%)	4.95 (3.80 – 8.27)	6.48 (4.21 – 7.72)	1.00
	maxEMGpara (μV)	95.65 (66.58 – 127.38)	77.27 (63.64 – 136.74)	0.841

Table 5 Within- and between-occasion repeat measurements of neural respiratory drive in healthy subjects. Data are shown as median (IQR)

To allow comparison of the relative repeatability of the raw *versus* normalised signal, Bland-Altman analyses were also performed expressing values as a percentage of the initial measurement. Wider 95% limits of agreement were noted for both intra- and inter-occasion measurements of EMGpara%max compared to EMGpara, although there was little difference in the bias values (Table 6).

		Expressed in original measurement units		Expressed as percentage of first measurement	
		Bias	95% limits of agreement	Bias	95% limits of agreement
Within-occasion (n=48)	EMGpara	0.63	-2.92 – 4.19	10.95	-52.61 – 74.52
	EMGpara%max	0.69	-4.72 – 6.09	8.56	-80.64 – 97.56
	MaxEMGpara	3.05	-78.40 – 84.49	2.18	-56.91 – 61.28
Between-occasion (n=19)	EMGpara	-0.21	-4.42 – 4.00	0.95	-67.88 – 69.77
	EMGpara%max	0.24	-4.81 – 5.29	-1.13	-73.28 – 71.02
	MaxEMGpara	0.81	-39.17 – 40.78	2.12	-36.25 – 40.50

Table 6 Results of Bland-Altman analyses of repeat measures of EMGpara and EMGpara%max

Discussion

This is the largest study to date examining factors determining EMGpara levels in healthy adult subjects. The results from this study indicate that sex difference is the most important determinant of EMGpara. A strong influence of anthropometric variables was not observed in this cohort. The results also indicate that normalising the raw EMGpara signal to that obtained during a maximal manoeuvre may not confer any benefit with respect to measurement repeatability and that the non-normalised signal may be of use.

Critique of the method

Previous studies (Murphy *et al.*, 2011; Reilly *et al.*, 2011; Steier *et al.*, 2011) including measurements of EMGpara in healthy individuals have recruited relatively small numbers of subjects from staff and students of respiratory physiology departments. The high level of understanding and extensive experience in performing respiratory function tests means that reference data derived from such cohorts cannot always be reliably extrapolated to the wider population. The substantially larger sample as well as the inclusion of naïve subjects suggests that the data from the current study will be more relevant when applying EMGpara to patient populations.

While the subjects included in this study were recruited from a variety of backgrounds and hence were broadly representative of the wider population, the median age was relatively low at 31 years of age. In addition, many of the older subjects studied were masters athletes (Pollock *et al.*, 2015), whose respiratory function may not be representative of the wider population of healthy older adults. Such factors may have masked any relationship between age and EMGpara, as has been demonstrated previously with EMGdi (Jolley *et al.*, 2009). Studying larger numbers of older individuals would help clarify any effects of age. The predominance of Caucasian participants in the current study population prevented the influence of ethnicity on EMGpara being examined.

Additional measures to BMI were made to quantify adiposity including bioelectrical impedance to assess overall body fat, waist-hip circumference to assess relative

distribution of fat, and neck circumference as a surrogate marker of upper body adiposity. None of these measures are, however, directly representative of chest wall adiposity, which is the main factor associated with attenuation of the EMGpara signal. In addition, the subjects within the current study were predominantly of a normal weight, with 20 (31.7%) classified as overweight (BMI>25) and only three (4.8%) obese (BMI>30). In subjects with elevated chest wall adiposity, attenuation of the EMGpara signal would be anticipated, but EMGpara%max would be expected to remain representative of respiratory load as both the resting and maximal EMGpara signals would be filtered to the same extent. We did not, however, observe any significant relationships between EMGpara or EMGpara%max and measures of adiposity. Elevated NRD resulting from the increases in respiratory load that occurs in obesity has been reported previously (Steier *et al.*, 2009). The presence and/or severity of respiratory complications of obesity are not however directly related to BMI, and may at least partly depend on the distribution of adipose tissue. Previous data have shown the increased work of breathing and altered lung volumes in obesity to be related to waist circumference (Steier *et al.*, 2014). Equally, the degree of EMGpara signal attenuation will not be directly related to body mass, but more specifically to the thickness of the upper chest wall fat layer. The current study was not able to quantify muscle-electrode distance directly. Future studies may benefit from using ultrasound to examine the relationship between chest wall skin and fat layer thickness and both raw EMGpara and EMGpara%max to determine the degree to which obesity both reduces the signal through filtering and results in its elevation due to increases in load. The small variability in such measures in addition to other influencing factors does however suggest that a very large sample size would be required. Conducting such large-scale studies with EMGpara is currently challenging due to the time consuming nature of manual EMG analysis.

Significance of the findings

These data provide limited reference ranges against which patient data from clinical studies can be compared and from which sample size calculations for future studies

can be undertaken. The results have also highlighted important methodological considerations when measuring EMG_{para}.

Most important of these is the effect of a mouthpiece on neural respiratory drive. While the increase in ventilation as a consequence of breathing via a mouthpiece or facemask has long been known (Gilbert *et al.*, 1972), the effect of this on EMG based indices of NRD has not to our knowledge previously been documented. Our data have quantified the magnitude of this effect. While ensuring methodological consistency within studies is always of utmost importance, the data objectively demonstrate that measurements obtained with and without a mouthpiece *in situ* cannot be viewed as interchangeable. The data also provide reference values for EMG_{para} measurements made both with and without a mouthpiece. Studies such as those involving cardiopulmonary exercise testing may however require the use of a facemask and although similar increases in EMG_{para} may occur, the magnitude of this increase should be specifically examined.

Sex differences in NRD during exercise have been previously documented, with female subjects shown to have higher EMG_{di} than males for equivalent minute ventilation (Schaeffer *et al.*, 2014). The increased level of NRD is attributed to sex based differences in respiratory system structure, such that after correcting for height women have lower lung volumes, narrower airways and lower respiratory muscle strength than men (Sheel & Guenette, 2008). Previous work has also indicated a greater ribcage muscle contribution to inspiration in women (Bellemare *et al.*, 2003). The current study adds to these previous findings by demonstrating the presence of sex differences in neural respiratory drive at rest.

Between-subject comparison of surface EMG data is known to be confounded by differences in muscle-electrode distance, as well as differences in skin composition and electrical impedance. Between-occasion comparisons can also be affected by subtle differences in measurement location, despite close attention to standardising electrode placement. Surface EMG measurements are therefore generally expressed relative to the EMG signal obtained during a maximal voluntary

contraction. The results from the current study have however suggested that such an approach may confer little advantage when quantifying EMGpara using the raw, non-normalised signal. Accuracy and consistency of electrode positioning for surface EMGpara is facilitated by the use of bony landmarks (positioning the electrode midway between the inferior border of the second rib and the superior border of the third, and measuring the distance from the sternum) and may therefore be more reproducible than surface EMG of large skeletal muscles. The smaller size of the parasternal intercostal muscles results in 19mm diameter surface electrodes covering a much larger proportion of the active muscle, making the signal more representative of the electrical activation of the whole muscle compared to measurements of large skeletal muscles.

The manoeuvres required for normalisation of the EMGpara signal may also result in recruitment of accessory inspiratory muscles such as the pectoralis major, located in close proximity to the muscle under study. This can result in substantial cross-talk, and acceptance of the maximal EMGpara signal obtained during a contaminated effort will result in a falsely low value for EMGpara%max. Conversely, achieving true maximal volitional activation of skeletal muscles can be challenging, even in well-motivated subjects. If full activation is not achieved, NRD as quantified by EMGpara%max will appear elevated, and subjects may appear to reach supra-maximal levels of NRD during conditions of heavy load, for example at end-exercise. Both of these normalisation errors result in misrepresentation of the respiratory load-capacity balance. In research settings, errors of this nature may preclude accurate and useful physiological insights. When considering clinical application of the technique, such errors would be unacceptable due to the potential for inappropriate diagnostic or treatment decisions.

We have shown somewhat greater variability of EMGpara%max compared to the raw signal alone, with wider limits of agreement for EMGpara%max both within- and between-occasion. These data suggest that use of EMGpara%max may not, therefore, confer the advantage with respect to repeatability that has previously been assumed. Using the raw EMGpara signal may allow the technique to be

applied in more diverse populations, particularly those unable to reliably perform the maximal volitional efforts required for normalisation such as young children or sedated patients receiving critical care. Recent data have demonstrated significantly higher raw EMGpara values in preschoolers with wheezing illness and children receiving intensive care than healthy youngsters (MacBean *et al.*, 2016).

The limits of agreement between repeat measures are somewhat higher than have been reported previously in healthy populations (Murphy *et al.*, 2011; Reilly *et al.*, 2011). As stated above, subjects in these earlier studies had experience of respiratory measurements and would therefore be expected to produce repeatable results. The use in the current study of an unselected cohort of predominantly naïve subjects would more likely represent performance of EMGpara measurements in clinical populations.

The limits of agreement from Bland-Altman analyses provide valuable insight as to what might be considered a significant change in neural respiratory drive. If considering clinical application of EMGpara-based measurements, however, detailed studies of repeatability using patient populations would be required to determine thresholds for significant change. It is likely that the effect of lung and chest wall pathology on the respiratory load-capacity balance may be of greater significance than the relatively subtle influences affecting NRD in healthy subjects in the current study. Variability of up to 4µV or 5% of maximum represents a much smaller proportion of the resting signal in patients with significant lung disease (Murphy *et al.*, 2011; Reilly *et al.*, 2011; Steier *et al.*, 2011) compared to the healthy subjects studied here. Of note, however, is the slightly higher within- than between-session variability, with higher first measurements within the same testing session. These data suggest that naïve subjects may perhaps require longer to acclimatise prior to commencing measurements of neural respiratory drive. In clinical populations, where concerns about diagnosis of or deterioration in a respiratory condition may be present, the effect of anxiety may be more notable and therefore have a more pronounced effect than that observed in the current study, though as stated above

the relative contribution of such anxiety over pathophysiological influences may not be substantial.

Conclusions

These data provide sex-specific reference values for EMGpara, EMGpara%max and NRDl. The data from the current study also support the use of the raw EMGpara signal to quantify NRD from the parasternal intercostal muscles, expanding the potential use of the technique beyond only individuals capable of reliably performing maximal inspiratory manoeuvres. These data substantially advance the potential use of surface electromyography of the parasternal intercostal muscles as a marker of respiratory load-capacity balance.

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